

Understanding surface-adsorption of proteins: the Vroman effect

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It is now well accepted that cellular responses to materials in a biological medium reflect greatly the adsorbed biomolecular layer, rather than the material itself. Here, we study by molecular dynamic simulations the competitive protein adsorption on a surface (Vroman-like effect), i.e. the non-monotonic behavior of the amount of protein adsorbed on a surface in contact with plasma as a function of contact time and plasma concentration. We show how the effect can be understood, controlled and inverted.

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When nanoparticles are in contact with blood plasma, or other biological fluids, biomolecules rapidly coat the bare surface in a relatively selective manner [1]. It is increasingly accepted that the early biological responses to nanoparticles will be determined by the adsorbed biomolecules rather than the pristine surface alone [2, 3]. Because of their size [2, 4] nanoparticles are trafficked by active transport processes throughout the organism, using the information from the protein sequences associated with the surface of nanoparticles. Unlike the situation for flat macroscopic surfaces say of medical implants, for nanoparticles the protein environment changes in different compartments of cells and organs, as the nanoparticle traffics. This has lent urgency to the modern interest in understanding the phenomenon at a more fundamental level [2]. Still, we can learn a lot from an understanding of the process for flat surfaces [5]. Studying the adsorption of Fibrinogen on a surface in contact with blood plasma, Vroman found that the surface concentration of Fibrinogen shows a maximum at an intermediate contact time, indicating that Fibrinogen is replaced with time by one or more families of different proteins [6]. The phenomenon is not specific to Fibrinogen, but is a general effect for many other proteins [7]. The plasma proteins compete for the occupation of the surface, resulting in a sequential competitive adsorption, known as the Vroman effect.

The effects depends on numerous factors such as the plasma dilution, the temperature, and the specific surface chemistry [8]. In highly concentrated plasma, the sequential adsorption takes place in seconds, but it takes several minutes when the plasma concentrations has physiological values [9]. The more hydrophobic the surface, the stronger is the protein adsorption, eventually inducing irreversible adsorption [10]. Here we study the effect by Molecular Dynamics (MD) of a model protein solution in contact with glass. We consider the three most abundant proteins in human blood: Albumin (Alb), Immunoglobulin- γ (IgG) and Fibrinogen (Fib), for which

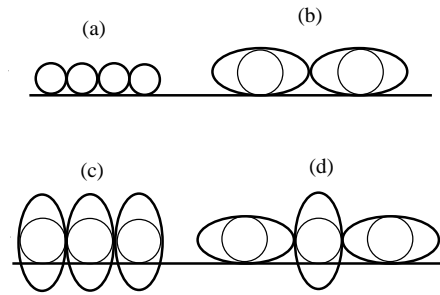


FIG. 1. Schematic representations of different proteins adsorbed on the surface. (a) Alb is a globular protein characterized by the radius R_A . In all the panels the continuous line represents the surface profile. IgG or Fib are represented as ellipsoids that interact with each other along the long axis $R_{S,i}$ (b), or along the short axis R_i (c). In the last case they are partially deformed (denatured) by the surface adsorption, represented here by a partial overlap of the ellipsoids with the surface. (d) At a random moment, the adsorbed ellipsoids can have different arrangements.

competitive adsorption has been observed [11]. We show that the sequence of adsorption can be explained in terms of the relative bulk concentrations, diffusivities and surface affinities of the proteins and that by thermal or chemical energy-depletion is possible to control and invert the effect.

Alb is a globular protein, with an almost spherical shape. We model Alb-Alb interaction as

$$V_A(r) = \left(\frac{2R_A}{r} \right)^{24} \quad (1)$$

where R_A is the radius of Alb and r the protein-protein distance (Fig. 1a). Attraction among protein is not included at this level of description, as it is small compared to protein-surface interaction [11]. Despite this rough zero-order approximation, our results support *a posteriori* this assumption within the approximations of our approach. IgG and Fib in their folded conformation have

non-spherical shapes. In particular, the IgG structure resembles a γ and the Fib resembles an elongated ellipsoid. They both can be approximated by ellipsoids with two principal axes of rotation along which they interact with each other (Fig. 1b-d). This is encoded through the protein-protein potential, within the same protein family,

$$V_i(r) = \left(\frac{2R_i}{r}\right)^{24} + \frac{3}{1 + \exp(30(r - 2R_{S,i})/2R_A)} \quad (2)$$

where the index $i = I, F$ stands for IgG (I) and Fib (F), with a hard-core radius R_i and a soft-core radius $R_{S,i}$ [13]. Interaction between pairs of proteins of different families are given by Eq.(2) with parameters R_i and $R_{S,i}$ equal to the averages of the corresponding parameters for each family, and with $R_A = R_{S,A}$ for Alb.

The protein-surface interaction is given by

$$V_{24,12}(r) = 4\epsilon_i \left(\left(\frac{\sigma_i}{r}\right)^{24} - \left(\frac{\sigma_i}{r}\right)^{12} \right) \quad (3)$$

where ϵ_i is the attractive energy between the surface and a protein of the family i , and $\sigma_i = R_i/2^{1/6}$, with $i = A, I, F$, is the maximum approach distance between each protein and the surface.

For each family of proteins i , we set the soft-core radius $R_{S,i} = R_H$ the hydrodynamic radius, determined experimentally from the diffusion coefficient D through the Einstein-Stokes equation $D = \frac{k_B T}{6\pi\eta R_H}$, where η is the viscosity of the medium, under the assumption that the proteins can be approximated by a sphere. The hard-core radii R_i are set by imposing for each protein that the experimental surface concentration corresponds to the close packing configuration [11]. These conditions give $R_A = 3.55$ nm, $R_I = 4.9$ nm, $R_F = 6.58$ nm, $R_{S,I} = 5.51$ nm and $R_{S,F} = 11$ nm. Protein masses $M_A = 67$ KDa, $M_I = 150$ KDa, $M_F = 340$ KDa, necessary to determine the time scales, are known from experiments [10]. Protein-surface attraction energy ϵ_i can be calculated from the adsorption rate constants [11]. These rates are proportional to the probability for a protein i to attach to the nearby surface

$$P_i \propto \exp\left(\frac{\epsilon_i}{k_B T}\right). \quad (4)$$

However, the ϵ_i in physical units are not known *a priori*. Hence, we consider the relative probabilities for different proteins $\frac{P_i}{P_j} \propto \exp\left(\frac{\epsilon_i - \epsilon_j}{k_B T}\right)$, from which is possible to determine the values of the different energies as

$$\frac{\epsilon_j}{\epsilon_A} = 1 - \frac{k_B T}{\epsilon_A} \ln\left(\frac{P_A}{P_j}\right) \quad (5)$$

adopting ϵ_A for Alb as the energy units. We set ϵ_A by comparing our simulations results with experiments at ambient temperature, and get $\epsilon_I = 2.79 \epsilon_A$ and $\epsilon_F =$

$6.08 \epsilon_A$. We express all the results in terms of the Alb units: $M_i^* \equiv \frac{M_i}{M_A}$ for the mass, $R_i^* \equiv \frac{R_i}{R_A}$ for lengths and $\epsilon_i^* \equiv \frac{\epsilon_i}{\epsilon_A}$ for the energies, $t^* = \left(\frac{R_A^2 M_A}{\epsilon_A}\right)^{\frac{1}{2}}$ for the time, $T^* = \frac{k_B T}{\epsilon_A}$ for the temperature, and $\Delta E/\epsilon_A$ for the energy changes of the buffer. In experiments ΔE is controlled by adding sodium azide, or other depletion-energy chemical agents, to the protein solution [12]. In the following we drop the $*$ for sake of clarity.

We perform MD simulations at constant T , constant volume V and constant number of proteins N_i , in a parallelepiped with two square faces and four rectangular faces. A square face is occupied by the attractive surface, the other by a wall interacting with the proteins through the repulsive part of the $V_{24,12}$ potential. We apply periodic boundary conditions (pbc) along the four rectangular faces. The volume concentrations of proteins is taken to match the average concentrations of the human plasma, with $c_A = 4.25$ g/dl, $c_I = 1.25$ g/dl and $c_F = 0.325$ g/dl, at $X_P = 100\%$ plasma concentration in blood. When a protein is adsorbed on (released by) the surface, we keep its bulk concentrations constant by inserting (deleting) a protein of the same family in a randomly-chosen empty (occupied) space of the box.

Experiments are usually carried out for highly diluted plasma, at concentration as small as $X_P = 0.1\%$, to slow down the adsorption rate to minutes or hours, allowing precise measurements. However, such low rates would decrease the statistics of our MD simulations. We, therefore, perform our simulations in conditions that are closer to those of practical interest, with X_P as high as 100%, 50% and 25%, by considering different sizes of the simulation box while keeping constant the initial number of proteins, their relative proportions, and the size of the adsorption surface.

For each X_P we average the results over fourteen independent runs, starting from independent initial configurations that have been equilibrated by applying pbc in any direction. We find that protein surface concentrations C_i^S are non-monotonic in time (Fig. 2). For any considered X_P , Alb is the first protein that reaches the surface, inducing an increase of C_A^S . When the second fastest and second most affine protein, IgG, diffuses to the surface, it displaces Alb, leading to a decrease of C_A^S and an increase of C_I^S . Finally Fib, which is the slowest and most affine protein to the surface, takes over decreasing C_I^S and increasing C_F^S . Each C_i^S saturates toward an equilibrium value at long times, while the total surface concentration of proteins is saturated at early times. This behavior qualitatively reproduces the Vroman effect, apart from the behavior of Fib that here is monotonic, while in experiments has a maximum due to the competitive adsorption with heavier and more surface-affine plasma proteins not included in our model [14]. The only effect of reducing X_P is a slowing down in the dynamics of the process, as observed in experiments [15].

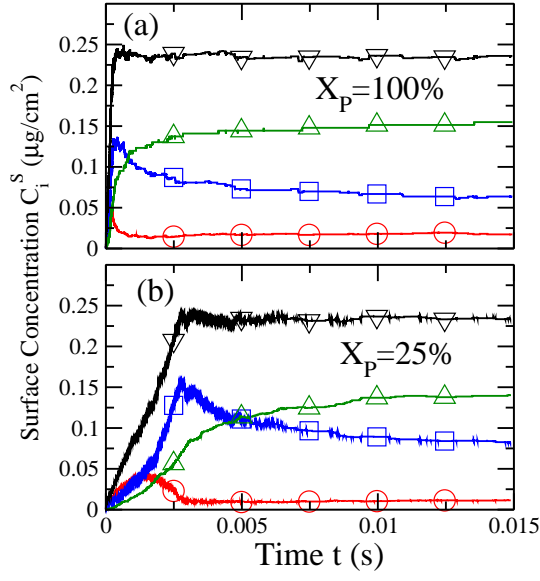


FIG. 2. Simulations at $T = 300$ K and (a) $X_P = 100\%$ and (b) $X_P = 25\%$ show that surface concentration C_i^S of Alb (○), C_I^S of IgG (□) and C_F^S of Fib (Δ) are non-monotonic with time, while their sum is (▽). At $X_P = 50\%$ (not shown) we find the same behavior with time-scales intermediate between those in (a) and (b). Bulk concentrations are as indicated in the text. Errors are smaller than symbol sizes.

The model allows us to understand the sequence of surface occupation as a consequence of the competition between the smaller, but less affine, proteins with the more affine, but bigger, proteins. For example, we test that by increasing the Alb affinity, or artificially setting to the same value all the diffusion constants, the effect disappears. Therefore, affinity and hydrodynamic radius are the relevant protein parameters for the effect.

Next, we study how energy depletion of the protein solution affects the sequence of adsorption. Here, for sake of simplicity, we decrease T , reducing the kinetic energy of the solution, but neglecting possible effects of the protein stability. We find (i) that, although the surface affinity of Fib is stronger than that for IgG, the latter becomes the dominant protein adsorbed on the surface for long time scales; (ii) that, by changing X_P , the time scale of the process becomes longer, but the inversion of the protein concentration is always present (Fig. 3). Hence, the energy depletion leads to an inversion of the Vroman effect.

By comparing the results at different energies, $k_B T$, and same X_P (Fig. 2-3), we observe only a weak energy-dependence of the times at which each C_i^S reaches its maximum. Hence, the time-scales of the process are mainly controlled by the total plasma concentration X_P .

Once we have understood that the protein layer covering the surface is controlled by the energy depletion of the system, it is interesting to ask if a sudden change of external conditions could induce a different composi-

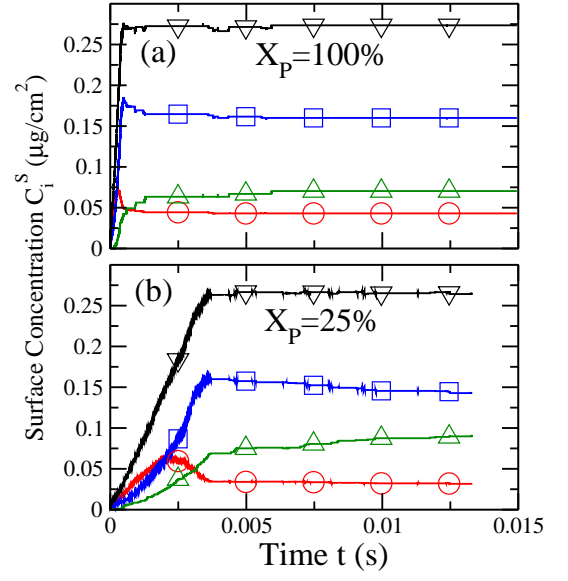


FIG. 3. Surface concentrations C_i^S as function of time for $T = 120$ K at (a) $X_P = 100\%$ and (b) $X_P = 25\%$. At long time, $C_I^S > C_F^S$, with an inversion with respect to the standard conditions in Fig. 2 where $C_F^S > C_I^S$. We find the same qualitative behavior at $X_P = 50\%$, not shown. Errors and symbols are as in Fig. 2.

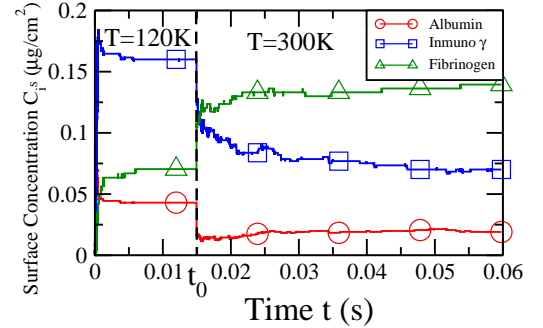


FIG. 4. The surface concentrations C_i^S , as a function of time for $X_P = 100\%$, is drastically affected when the system undergoes a sudden change from an energy-depleted condition to a normal condition. The vertical dashed line marks the time t_0 of the change. We control the energy of the solution by changing the external parameter T from $T = 120$ K to $T = 300$ K. Errors and symbols are as in Fig. 2.

tion of this layer, determining different biomimetics surface properties. This situation could occur, for example, when a medical device is manipulated in a bioenvironment whose composition is externally controlled during a surgery [18]. In particular, we study the case in which the system is first equilibrated under energy-depleted conditions and subsequently undergoes a sudden change that reestablishes the normal conditions (Fig. 4).

At short times the energy-depleted system evolves until the equilibrium concentrations are reached. Under these conditions, as discussed (Fig. 3), the dominant protein

is IgG instead of Fib. At time t_0 we switch to normal conditions, forcing the system out of equilibrium. As a consequence, the system re-enters a transitory situation in which the concentrations C_i^S evolve until they reach their new equilibrium values at long times. In the specific case considered here, we observe a fast change in the surface concentrations, with C_F^S of Fib overcoming C_I^S of IgG, being the first, under normal conditions, more stable on the surface than the second. The final equilibrium concentrations are reached at large times. We observe also a sudden change in C_A^S of Alb, between the two equilibrium concentrations characteristics of the two values of the external parameters T . However, C_A^S always equilibrates to a value that is smaller than C_I^S and C_F^S , consistent with its long-time values in Fig. 2-3. By decreasing X_P , we find the same qualitative behavior for a sudden energy-change, but with the transient regime extending to longer times, consistent with Fig. 3. Hence, at experimental values of X_P the switching behavior would occur on time scales that are comparable to those characteristic of the Vroman effect.

We remark that our predictions about inverting the Vroman effect by changing the experimental control parameters should hold only if the protein adsorption on the surface is reversible. If the adsorption is, instead, irreversible the change of external parameters should not lead to a new composition of the protein layer. Indeed, under many practical conditions of interest for blood plasma, it would appear that the binding is indeed mostly irreversible [2, 16]. Hence, the switching protocol proposed above represents a possible experimental way to evaluate how strongly irreversible is the adsorption process on a specific surface.

For an irreversible adsorption process, our findings predict that by appropriately controlling the parameters of the protein solution, such as the amount of depleted energy, it is possible to engineering a specific biomimetic covering of a surface. Due to the irreversibility, the proteins, once adsorbed, cannot desorb from the surface, even if the external conditions are modified. Therefore, it is feasible to cover a device surface with any desired protein composition, targeted to a specific biomimetic property, by selecting an appropriate initial condition. Subsequently, the device could be used under physiological conditions with no further changes of the protein cover and its biomimetic properties.

In conclusion, we study, by MD simulations of a coarse-grained model, the Vroman effect for three kinds of proteins: Alb, IgG and Fib. We show that the effect is the consequence of the differences among the proteins properties (mass, size and affinity) which regulate their diffusive behavior and their interaction with the surface. These differences lead to a process of competitive adsorption

on a surface, in which the different families of proteins occupy sequentially the surface, replacing each other, until an equilibrium situation is reached. By decreasing the total concentration of protein in the solution, keeping the relative concentrations fixed, the time scales of the process increase and the maxima of surface concentration for each family of proteins occur at longer times.

We find that the protein surface concentrations at equilibrium depend on external control parameters. In particular, we find that energy depletion induces a drastic change in the composition of the covering protein-layer, leading to an inversion of the Vroman effect. Our results show that the inversion can be used to quantify how strongly irreversible is the process of surface adsorption of the proteins, an information useful in studies of thromboembolic events [17]. Furthermore, these results suggest the possibility of engineering the composition of the protein layer covering a surface in a controlled way, a feature particular relevant in biomimetic applications.

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